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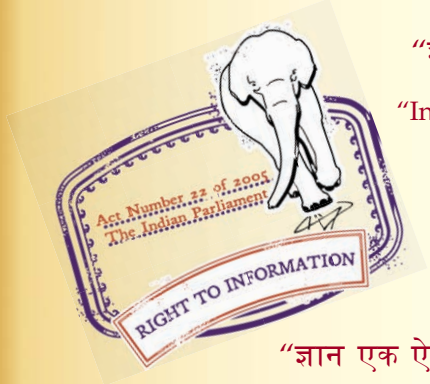

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GUIDE FOR STERILIZATION OF MEDICAL PRODUCTS

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BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
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TO
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(*Page* 4, *clause* 3.2.1.3, *second line*) — Delete the word 'personnel'.

[*Page* 5, *clause* 3.5(iv)] — Substitute 'Posses' *for* 'Process'.

(*Page* 6, *clause* 3.6, *third sentence*, *seventh line*) — Substitute the following for the existing sentence:

'These discs or strips are placed alongside in the packets most difficult to sterilize in a sterilization chamber.'

(*Page* 7, *clause* 4.1.4, *second line*) — Insert 'ATCC No. 7953 or 12980/NCTC No. 10007' after '*Bacillus stearothermophilus*'.

(*Page* 8, *clause* 4.2.1, *second line*) — Insert 'ATCC No. 9372' after '*Bacillus subtilis*'.

(*Page* 9, *clause* 4.4.1.1, *third line*) — Substitute '1.33 MeV' *for* '1.33'.

(*Page* 9, *clause* 4.4.1.1, *fifth line*) — Substitute 'radioisotope' *for* 'isotope'.

(*Page* 10, *clause* 4.4.4, *last sentence*) — Substitute the following for the existing:

'Duration of radiation sterilization process depends upon the activity of the radioisotope source.'

(*Page* 10, *clause* 4.4.4, *Note*, *second line*) — Delete the word 'standard'.

(*Page* 10, *clause* 4.4.5, *first line*) — Substitute 'kGy' *for* 'KGy'.

(*Page* 10, *clause* 4.4.6, *second sentence*) — Substitute the following with the existing:

'Some suitable systems for routine dosimetry are:

i) Dyed clear perspex,
ii) Radiochromic dye films,
iii) Ceric-cerous sulphate solution, and
iv) Ethanol-cheorobenzene solution'.

(*Page* 10, *clause* **4.4.8**, *second line*) — Insert '/ATCC No. 14884 or NCTC No. 8241' after 'ATCC No. 27142'.

(*Page* 10, *clause* **4.4.9**, *ninth line*) — Insert 'surgical' after 'as'.

(*Page* 17, *clause* **B-2.1**, *sixth line*) — Insert '/ATCC No. 9372' after 'NCTC 10073'.

(MHD 14)

Reprography Unit, BIS, New Delhi, India

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GUIDE FOR STERILIZATION OF MEDICAL PRODUCTS

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(*Continued on page* 2)

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GUIDE FOR STERILIZATION OF MEDICAL PRODUCTS

0. FOREWORD

0.1 This Indian Standard was adopted by the Indian Standards Institution on 24 December 1981, after the draft finalized by the Surgical Instruments Sectional Committee had been approved by the Consumer Products and Medical Instruments Division Council.

0.2 This guide has been prepared to give knowledge to the manufacturers and consumers of surgical items for various methods adopted for sterilization and tests of sterility.

0.3 ISOMED Section of Bhabha Atomic Research Centre, Bombay has considerably helped for preparing this guide.

0.4 In preparing this standard considerable assistance has been derived from ISO document No. ISO/TC 150/SC 1 N 26 'Sterilization of medical devices', issued by the International Organization for Standardization; and Indian, US and British pharmacopoeiae.

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1. SCOPE

1.1 This guide prescribes the guidelines for sterilizations of medical products and method of tests for sterility.

2. TERMINOLOGY

2.1 The definitions as given in **2.2** shall apply.

2.2 Sterilization — Sterilization is a process by which the probability of occurrence of a viable micro-organism in a medical product is reduced to less than 10^{-6}. The terms partial and complete sterilization are not correct.

3. GENERAL

3.1 Efficiency — The efficiency of any given sterilization process is influenced by the nature of product, the extent and type of any contamination present, which depends very much on the conditions under which

the product has been prepared. The principles of good manufacturing practice should be observed in the manufacturing of products.

3.2 Bioburden — The products to be sterilized shall be as free as possible from microbial contamination (generally lower the contamination, greater is the margin of safety). No figures can be attached to this qualitative statement as the efforts to reduce the level of bioburden should be a continuous process.

NOTE — Emphasis should be placed on the development of the highest possible standards of manufacturing conditions involving clean rooms, filtered air, protective clothing for operators and above all, discipline on all hygiene matters and for the routine control of raw materials known to be subject to contamination. The manufacturer should periodically monitor the products, prior to sterilization, to assure that the microbial bioburden, established on validation has not significantly changed. Micro-organisms recovered from the product and the environment should be characterised, and a record should be maintained.

3.2.1 The bioburden determination should be carried out aseptically (for example, by working on a laminar air flow bench) so that the risk of laboratory contamination is avoided. For every batch of a product the following number of units should be drawn for testing:

No. of Units in a Batch or Lot	*Sample Size*
100 or less	10 percent and not less than 4
101 - 1 000	2 percent and not less than 10
More than 1 000	Not less than 20

3.2.1.1 The medical products are rinsed with sterile saline containing 0·05 percent polysorbate. An aliquot of the solution is then filtered through a membrane filter (0·45μm). The filter is spread on a suitable sterile culture medium in a petri dish and incubated for a minimum of 96 hours. Observations are made at the end of every 24 hours. The number of growing colonies, multiplied by the dilution factors indicates the number of a colony forming units (CFU) present in the product. The types of micro-organisms isolated should be identified at least up to the genus stage.

3.2.1.2 In evaluating the test results, the average CFU per unit of product, known from experience to be attainable in similar articles, shall be taken into account. For example, if the average CFU per hypodermic needle, syringe and infusion set is 10, 50 and 500 respectively, and if the CFU in any later determinations exceeds these figures significantly, the manufacturer shall find the cause of this increase, and initiate improvements in the hygiene of processing, handling and packaging.

3.2.1.3 The test shall be carried out by personnel skilled and experienced in aseptic working procedures personnel under the supervision of an experienced microbiologist. The tests shall not be carried out

under direct ultraviolet light nor should the products be exposed to ultraviolet light. The test shall be carried out by the manufacturer on every batch of product and the records on the number of types micro-organisms in that batch shall be maintained.

3.3 Requirements of Sterilization — The following are the requirements for sterilization process:

i) Vigilant supervision of equipment, instruments and procedures for operation;

ii) Maintenance and calibration by personnel well trained in devising and applying methods for attaining sterility;

iii) Adequate proof of the effectiveness of the procedures used;

iv) Decontamination of recirculation products of bacteria and the resistance of the contaminant bacteria. Adequate removal of substances which may interfere with direct contact of steam or other media, for example, oils, greases, protein, soap, blood, tissue debris, etc; and

v) Identification on the primary packing material of adequate sterilization by a chemical indicator. The choice of appropriate process requires knowledge and experience concerning the sterilization process.

3.4 Process Effects — The effect of the chosen sterilization procedure on the product (preferably including its primary-package and final container) shall be validated before that procedure is applied in practice. Failure to follow a process meticulously involves the risk of a non-sterile or deteriorated product.

3.5 Packaging — Sterilization of medical products in their primary packaging and final container is advantageous and beneficial. The material employed for packaging the products shall, have the following characteristics:

i) Shall allow penetration of the sterilizing agents;

ii) Prevent entry of bacteria, during cooling and storage;

iii) Robust enough to withstand handling, transportation, moisture and heat;

iv) Process adequate bursting strength under conditions of sterilization; and

v) Permit egress of air and filter entering air during and after sterilization respectively.

Hard objects such as syringe, valves and prostheses shall have rounded edges because sharp edges perforate the packaging film under impact. The package shall be sealed so that the seal width is 2 to 3 mm. The body of the packaging material as well as the seal shall be free from defects and leakage.

3.5.1 The primary packaging shall be free from defects, such as pinholes, leakage, delamination, etc, which shall affect its function. It shall also be unaffected when dropped as described in **3.5.2**. A method for determining the presence of pinholing in plastics film shall be as given in Appendix A. The leakage shall be tested as described in **3.5.3**.

3.5.2 *Drop Test* — When dropped from a height of 1 metre on to a shock-resistant surface the primary packaging shall be undamaged and the sterile conditions of the package unchanged. The primary packaging shall show no sign of leakage when tested as described in **3.5.3**.

NOTE — The drop test is only to ensure that the packaging film has sufficient impact strength and that the product does not have sharp edges or spikes to damage the film. As the product may break in the drop test, it should not be understood that the plastics primary packaging should protect the product from mechanical damage.

3.5.3 *Leakage Test* — Place the product in primary packaging, weighed if necessary, under 5-10 cm of water in a vacuum desiccator or any other suitable vessel and evacuate the space above the water surface to a vacuum level of 250 mm Hg. Closely observe the primary packaging for any localized stream(s) of gas bubbles during the next 5 minutes. Then restore atmospheric pressure to the space above the water surface and, after 2 minutes remove the primary packaging from the water. Dry the external surfaces and examine for evidence of water penetration.

NOTE — As a refinement of this procedure, a small amount of fluorescin may be dissolved in the water after which the primary packaging is examined under ultraviolet light for evidence of water penetration,

3.6 Biological Indicators — Biological indicators are standardized preparations of specific microorganisms relatively resistant to the particular sterilization process, and are used to demonstrate in a positive manner the adequacy of the sterilization process. An aliquot (containing about a million spores) of a viable culture of a known species of microorganism is added to disks or strips of filter paper or any other suitable carrier. These disks or strips are placed alongside in the packets in a sterilization chamber. The preparation of stock suspensions of selected microorganism requires competence in the techniques for maintaining and standardizing the microbial culturers. Where spore suspensions are used to prepare a biological indicator, it is important that the stock suspension contain predominantly spores that have been held in non-nutritive liquid. Precautions are necessary to ensure that the

biological indicator inoculum contains no substances that substantially alter the resistance of the indicator organisms to the sterilization process. It is important that the biological indicator preparations commercially available maintain their label concentration of viable spores or cells throughout the dating period, since the performance of the biological indicator is a function of its initial viable count as well as the resistance of each viable unit or cell. Preparations made by the user should have equivalent properties.

4. METHODS OF STERILIZATION

4.1 Steam Sterilization — This process is ordinarily carried out in an autoclave and employ steam under pressure. The following combinations of temperature and time are appropriate for sterilizing by heating in an autoclave:

Holding Temperature	*Minimum Holding Time* (min)
115° to 118°C	30
121° to 124°C	15
126° to 129°C	10
134° to 138°C	3

4.1.1 The prescribed conditions are those to be maintained throughout the load during the holding period of the sterilization cycle. In as much as the process depends upon the presence of moisture as well as elevated temperature, adequate measures shall be taken to ensure that all air has been removed from the chamber prior to the initiation of the sterilization cycle.

4.1.2 Information on the physical conditions within the autoclave, may be obtained by inserting temperature sensitive elements into representative container and with additional elements at the previously established coolest part of the loaded chamber. It is desirable that each sterilization cycle be recorded on a temperature time chart.

4.1.3 *Chemical Indicators* — The chemical indicators, such as tapes are employed to detect the conditions of sterilization in the chamber.

4.1.4 *Biological Indicator* — Disks or strips containing about a million spores of suitable strains of *Bacillus stearothermophilus* are utilized as biological indicators to prove the adequacy of the sterilization process. The approximate D_{10} value may be 1½ minutes for saturated steam at 121°C.

NOTE — D_{10} value means, the time in which 90 percent of the organisms are expected to be killed.

4.2 Sterilization by Dry Heat — The process of sterilization is done by dry heating in suitable chamber using a suitable temperature, time combination, for example, 160°C for 2 hours or 180°C for 30 minutes.

NOTE — The sterilization period is reckoned from the time when all parts of the sterilization material have assumed the sterilization temperature.

4.2.1 The efficiency of the dry heat sterilization procedure shall be checked by means of test duration of *Bacillus subtilis* prepared for the purpose of checking the dry heat sterilization procedure.

4.3 Sterilization by Ethylene Oxide — The material to be sterilized is exposed either to pure ETO or to a mixture of ETO with an inert gas (CO_2 or suitable fluorinated hydrocarbons). This process is difficult to control, the gas itself being toxic and when mixed in certain proportions with air, becomes explosive. Careful determination of the appropriate cycle parameters must be made as the level of humidification, heat gas concentration, and time of exposure relate directly to the product design (including penetrability, packaging system employed, bioburden of the product, and state of hydration of the micro-organisms).

4.3.1 *Bioburden* — A bioburden history of the product should be established since this factor influences the effectiveness of the sterilization process.

4.3.2 *Packaging Materials* — These shall be highly permeable to ETO, its diluents and air. Double thickness muslin or paper, or single thickness (up to 75 μm) polyethylene (PE) are materials of choice. Bags of nylon film, cellophane, saran, polyvinyl chloride (PVC) film and aluminium foil must not be used because of inadequate permeability. Laminates, such as cello-PE, mylar-PE and foil-PE may only be used, if the other side of the package is either uncoated paper or PE.

4.3.3 *Sterilization Process* — ETO sterilization requires specialized equipment designed for the particular purpose. The instructions provided by the manufacturer of the ETO sterilizer must be closely followed. The exposure conditions normally used with ETO mixtures at a temperature of 55°C and relative humidity (RH) 60 percent are given in Table 1. Before using an ETO gas cylinder for routine sterilization, the concentration of the gas must be assayed. The procedure and other detail of ETO sterilization shall be as given in Appendix B.

4.4 Sterilization by Ionizing Radiation

4.4.1 Electron accelerators and radioisotopes are two practical sources of radiation. Electron accelerators produce high energy electrons with a low penetrating ability and their use requires careful control of many variables affecting sterilization efficiency. Amongst these variables are electron energy, electron current, scan width, exposure time and product

density. Van be graff machines produce 1 to 3 MeV electrons. Linear accelerators produce 3 to 15 MeV electrons. Beam energies in excess of 9 MeV are not used as short-lived radioactivity is induced in the material. Owing to high dose rate, sterilization is completed in a fraction of a minute. The product thickness should be carefully controlled as the penetration of electrons is limited.

TABLE 1 EXPOSURE CONDITIONS USED WITH ETHYLENE OXIDE MIXTURE AT A TEMPERATURE OF 55°C, RH 60 PERCENT, AND DWELL PERIOD OF 60 MINUTES

(*Clauses* 4.3.3 and B-1.1)

Mixture Content (Percent)	ETO Concentration (mg/L)	Chamber Pressure (KN/m^2)	Minimum Exposure Periods (h)
(1)	(2)	(3)	(4)
10 ETO, 90 CO_2	450	200	6
20 ETO, 80 CO_2	670	125	4
	920	210	3
11 ETO, 54 Trichloro-fluoromethane, 35 Dichloro-difluoromethane	450	35	5
	850	125	3
12 ETO, 88 Dichloro-difluoromethane	650	50	4

4.4.1.1 *Radioisotope sources* — Two radioisotope sources cobalt-60 (half life = 5·26 years, gamma rays of 1·17 MeV (100 percent) and 1·33 (100 percent) and Caesium-137 (half life = 30 years, gamma rays of 0·66 MeV (95 percent), shall be employed for sterilization purpose. The cobalt-60 isotope is currently the most widely used source of gamma radiation. As these radiations have greater penetrating power than electrons, they are useful for bulky products.

4.4.2 *Bioburden* — It is desirable to establish the bioburden history of the product since this factor influences the effectiveness of the sterilization process.

4.4.3 *Packaging Materials* — A wide variety of reasonably thick (more than 75 μm) packing materials (polyethylene, polyvinyl chloride, polystyrene, laminates of paper-PE, cello-PE, mylar-PE, foil-PE, etc), may be employed.

4.4.4 *Sterilization Process* — The radiation sterilizers are operated according to the manufacturer's instructions. The process may be a batch type (with radioisotope sources), or a continuous type (with radioisotope sources or with electron beam generators). The entire

process is automatic and precisely controlled. Owing to a relatively low dose rate the sterilization may last from 8 to 100 hours depending on the strength of the source.

NOTE — The greatest advantage of the gamma ray sterilization process is that the products stacked in the final sealed, tamper-proof, standard, corrugated cartons may be sterilized.

4.4.5 *Dose* — A dose of 25 KGy (2·5 Mrad) is generally accepted as adequate for the purpose of sterilization, although other dosage levels may be employed provided that they have been authorized by the appropriate authority. Lower doses can be used only if (i) the products have low or susceptible bioburden, (ii) they are radiation sensitive, and (iii) additional controls such as microbiological monitoring of the product before and after exposure are carried out to assess the adequacy of the process.

4.4.6 *Dosimetry* — The radiation dose received by an irradiated product is measured by means of suitable chemical or physical methods, that are independent of dose rate. Some suitable systems for routine dosimetry are: (i) solutions of ferrous sulphate (up to 0·4 KGy), and (ii) acrylic plastics without or with sensitive dyes for example, perspex '*HX*' and red perspex respectively (up to 40 KGy).

4.4.7 *Chemical Indicator* — Chemical indicators, such as tapes are employed which by change of colour indicates the condition of sterilization.

4.4.8 *Biological Indicator* — Disks or strips containing about a million spores of suitable strains of *Bacillus pumilus* (for example E601 or ATCC 27142) are utilized as biological indicators to monitor the sterilization process using gamma or other ionizing radiation. The approximate D_{10} value may be 0·2 Mrad (for wet preparations) and 0·15 Mrad (for dry preparations).

4.4.9 *Precautions* — Repeated exposure to ionizing radiation is likely to induce a significant degradation in materials such as natural and synthetic fibres and plastics. With some materials, for example, cellulosic materials, major degradation occurs if they are autoclaved after they have been exposed to a sterilizing dose of gamma radiation. There is also the possibility of toxicity developing in certain products (for example, PVC) if they are exposed to ETO after previous irradiation. Therefore no attempt should be made to resterilize materials, such as dressings, plastics, etc, which have been previously sterilized by exposure to ionizing radiation.

5. STERILITY TESTS

5.1 The sterility tests presented herein are suitable for revealing the presence of viable forms of bacteria, fungi and yeasts in or on articles which have gone through the process of sterilization. Alternative procedures or procedural details may be employed to demonstrate that an article is sterile, provided the results obtained are at least of equivalent reliability.

NOTE — All temperatures herin specified are in Celsius degrees and with respect to autoclaves, generally mean the exhaust-line temperatures. Where a difference appears or in the event of a dispute, when evidence of microbial contamination is obtained by the procedure given in this text, the result so obtained is conclusive of failure of the article to meet the requirements of the test.

5.1.1 Adventitious microbial growth that is transmitted to an article or to inoculated test culture media from the environment during the course of a sterility test invalidates the results of the test. Hence, it is necessary to demonstrate that the proper precautions have been taken to exclude extraneous micro-organisms throughout the test period.

5.1.2 Where a sterility test is applied to discrete units drawn from a group of similar units, the results obtained, if negative, indicate, that the probability of the presence of contaminated units in the untested portion is below a level detectable by the sampling plan employed. No sampling plan for applying sterility tests to a specified proportion of discrete units selected from a sterilization load is capable of demonstrating with complete assurance that all of the untested units are in fact sterile. A further review of validation of sterilization is presented in the preceding clauses on various methods of sterilization.

5.2 Media — Media for the tests may be prepared as described in Appendix C, or dehydrated mixtures yielding similar formulations may be used provided that when reconstituted as directed by the manufacturer or distributor, they have growth-promoting properties equal or superior to those obtained from the form as given therein.

5.3 Growth Promotion Test — Test each autoclaved lot of medium for its growth promoting qualities, in the following manner. Inoculate duplicate test containers of each medium with less than 100 of the micro-organisms given in Table 2 and incubate according to the conditions specified for it.

5.3.1 The test media are satisfactory if evidence of growth appears within 7 days. The tests may be conducted simultaneously with the use of the test media for sterility test purposes, provided however, that the sterility test is considered invalid if the test medium shows no growth response.

5.3.2 Confirm the sterility of each lot of medium by incubations of representative containers, at the temperature and for the length of time specified in the test.

5.3.3 If freshly prepared media are not used within two days, store them in the dark, preferably at 2° to 25°. Finished media may be stored in unsealed containers for more than 10 days, provided that they are tested weekly for growth promotion. If stored in suitable sealed containers, the media may be used for not more than one year, provided they are tested for growth promotion every three months.

TABLE 2 DETAILS OF TEST MICRO-ORGANISMS

(*Clause* 5.3)

Sl No.	Medium	Test Micro-organisms	Incubation Temp (°C)
(1)	(2)	(3)	(4)
i)	Fluid thioglycollate	a) *Bacillus subtilis* (ATCC No. 6633)*	30°-35°
		b) *Candida albicans* (ATCC No. 10231)	30°-35°
		c) *Bacteroides vulgatus* (ATCC No. 8482†)	30°-35°
ii)	Alternative thioglycollate	a) *Bacteroids vulgatus* (ATCC No. 8482)	30°-35°
iii)	Soyabean casein digest	a) *Bacillus subtilis* (ATCC No. 6633)	20°-25°
		b) *Candida albicans* (ATCC No. 10231)	20°-25°

*If a spore forming organism is not desired, use *Micrococcus luteus* (ATCC No. 9341) at the incubation temperatures indicated in the table.

†If a spore forming organism is desired, use *Clostridium sporogenes* (ATCC No. 11437) at the incubation temperature indicated in the table.

5.3.4 *General Procedure* — Because of diversity in the nature of articles to be tested and other factors affecting the conduct of the sterility test, it is important to observe the considerations in performing sterility tests as given in **5.3.5** and **5.3.6**. For purified cotton, gauze, surgical dressings and related pharmacopoeial articles, open the package or container aseptically, and test the articles as described in Appendix D.

5.3.5 *Selection of Test Specimens and Incubation* — The quantity of information provided by sterility testing is related to the number of units tested. Test 20 units of the article with each medium. Incubate the test mixture for 14 days with fluid thioglycollate medium or alternative thioglycollate medium where so indicated at 30° to 35°C, and with soyabean casein digest medium at 20° to 25°C.

5.3.6 *Test Procedures for Direct Transfer to Test Media* — Sterility tests are applied to individual discrete units or to composites of such units. The number of units specified is chosen only for practical considerations of the required laboratory manipulations in performing the referee tests. As previously noted in the introductory paragraphs, results obtained in examining a discrete unit or units for sterility cannot be extrapolated with complete certainly to characterise the sterility of the untested portion of the lot.

5.4 Test Procedures Using Membrane Filtration Technique — Certain devices may be appropriately tested for sterility of the critical pathways by the membrane filtration technique. The manipulations additional to those employed in conducting the sterility test by direct transfer may be a potential source of extraneous contamination. The frequent use of nagative controls is highly recommended. The test procedure is described in Appendix E.

5.5 Observation and Interpretation of Sterility Test Results — At intervals during the incubation period and at its conclusion, examine the contents of all the vessels for macroscopic evidence of microbial growth, such as the development of turbidity. If no evidence of growth is found, the material tested meets the requirements of the test for sterility. If evidence of microbial growth is found, the material tested fails to meet the requirements of the test for sterility, unless it can be demonstrated by retests or by other means that the test was invalid for causes unrelated to the article.

In view of the possibility that microbial growth observed in the test was due to inadequate aseptic sampling and testing technique rather than to intrinsic contamination of the article, the following retests are permitted.

5.5.1 *First Retest* — The number of specimens selected, the volumes to be tested, and the media are the same as those indicated for the original sterility test. If no evidence of microbial growth is found, the material tested meets the requirements of the test for sterility. If microbial growth appears in this first retest isolate and characterise the microbial contaminate(s) of the first retest and compare with the contaminant(s) of the original sterility test. If the contaminant(s) cannot be differentiated readily, the material tested fails to meet the requirements of the test for sterility. If the contaminant(s) can be differentiated readily, a second retest may be performed.

5.5.2 *Second Retest* — The number of specimens selected is double the number tested in the original sterility test and in the first retest. The volumes tested from each specimen and the media are the same as those indicated for the original sterility test and the first retest.

5.5.2.1 If no evidence of microbial growth is found, the material tested meets the requirements of the test for sterility. If growth appears in this second retest, the material tested fails to meet the requirements of the test for sterility.

APPENDIX A

(*Clause* 3.5.1)

DETECTION OF PINHOLE IN PACKAGING FILM

A-1. REAGENTS

A-1.1 Ammonia Sensitive Solution — Dissolve 100 g of tartaric acid in 600 ml of water, add 15 ml of 60 percent w/v aqueous ferric chloride ($FeCl_3,6H_2O$), 120 ml of 10 N ammonium hydroxide and 500 ml of 12 percent w/v potassium ferricyanide. This solution is stable for several weeks when stored in the dark. Sheets of white, semi-absorbent paper are coated on one side with the reagent and then dried in the presence of light.

NOTE — The sheets at first are an olive-green, but upon exposure to light the ferric ions are reduced to ferrious which then react with ferricyanide to give the blue pigment ferrous ferricyanide (Turnbull's blue). This pigment is decomposed by excess ammonia to white ferrous hydroxide and ammonium ferricyanide.

A-2. APPARATUS

A-2.1 The apparatus shall consist of a circular polymethyl methucrylate cell divided into two compartments by the test specimen (*see* Fig. 1). The dimensions of the cell shall not be critical. The outlet from the top of the cell shall be connected by rubber tubing and glass T-pieces to a mercury manometer and a vacuum pump. The vacuum applied to the top compartment shall be controlled by an adjustable hose clip downstream from the manometer. The bottom compartment of the cell shall be connected on one side to a Drechsel bottle partly filled with concentrated ammonium hydroxide and on the other side to the vacuum line downstream from the hose clip. This flow rate may be achieved by adjustment of a second hose clip on the outlet line from the cell, or by inserting a capillary of suitable dimensions in the line.

A-3. PROCEDURE

A-3.1 The test specimen shall be placed on the flange of the cell and covered with the moistened reagent paper so that the reagent coated side faces the specimen. With coated materials, the coating on the specimen

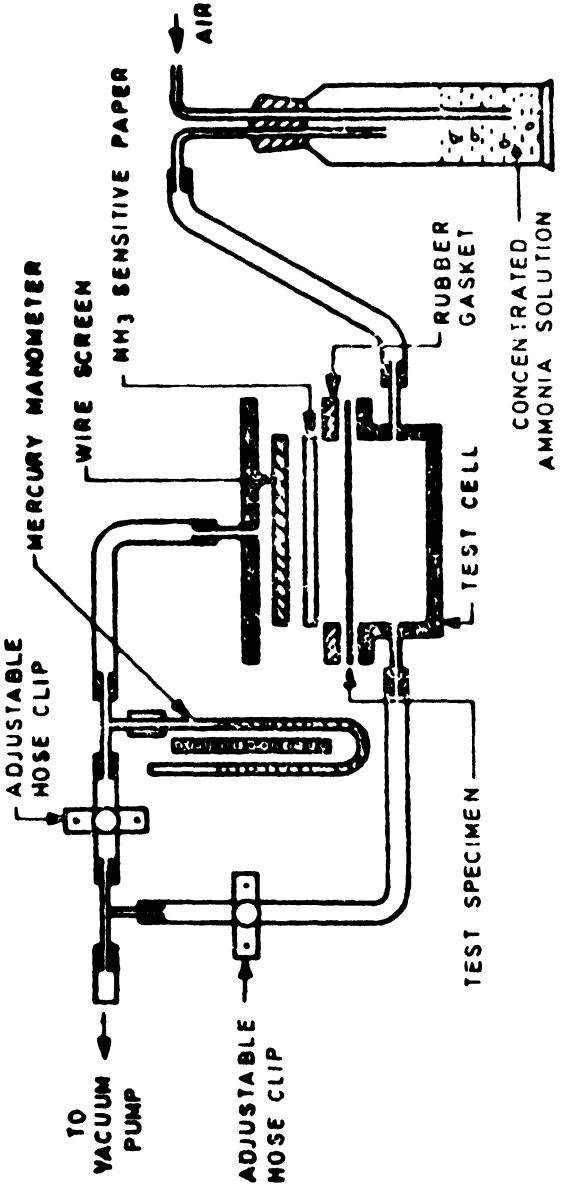

FIG. 1 SCHEMATIC DIAGRAM FOR APPARATUS FOR DETECTING PIN HOLES IN PACKING FILMS

shall face the reagent paper. A neoprene gasket shall be positioned on the paper, and a metal screen having a diameter approximately 2 mm less than the internal diameter of the cell and gasket shall be fitted.

A-3.2 The screen shall be used to prevent the moistened paper sealing into the flat surface of the cell cover when a vacuum is applied. To prevent distortion of the specimen the thickness of the screen and gasket should be similar. The cell cover shall then be fitted and clamped by four spring-back paper clips. With the water pump turned on, the hose clip is adjusted to give a vacuum of approximately 200 mm Hg shall then be in the top of the cell. The tubing to the ammonia supply shall be connected and ammonia drawn through the bottom of cell shall approximately be 150 ml/min. This flow rate may be achieved by adjusting the clip on the outlet line from the cell or by inserting a suitable capillary in the line. At the end of the test period the ammonia line shall be disconnected and air shall be drawn through the cell for approximately 30 seconds to remove residual ammonia. The cell is then dismantled and the sensitized paper examined for white spots, which indicate discontinuities in the test specimen.

NOTE — The time required for a test at a specific vacuum level depends on the nature of the film material. The optimum test time is one that gives small, sharply defined spots on the sensitized paper, and should be predetermined for each type of test material. Test times that are inconveniently short or long may be adjusted by decreasing or increasing, respectively, the vacuum on the top of the cell. However, when long test times are used, ammonia may permeate through an organic film, free of pores by the solution diffusion mechanism, resulting in a uniform discoloration of the reagent paper. Permeation of gases by this mechanism is independent of the total pressure and dependent only on the partial pressure difference of gas across the specimen, whereas diffusion through pores is dependent on total pressure, hence the rate of diffusion of ammonia through pores can be controlled by the vacuum applied on the top of the cell.

APPENDIX B

(*Clause* 4.3.3)

PROCEDURE AND OTHER DETAILS OF ETO STERILIZATION

B-1. PROCEDURE OF STERILIZATION

B-1.1 It is the general practice to place the material in the chamber previously heated to 55°C. An initial vacuum of approximately 686 mm Hg is drawn. Since too rapid reduction of pressure within the chamber may cause sealed bags to burst, the evacuation is to be carried out gradually. Moisture is then introduced to provide relative humidity (RH) of 40 to 60 percent. After a dwell period of 60 minutes to allow

hydration of spores, the ETO mixture is then introduced to the pressure required to give the desired concentration of ETO as given in Table 1. This pressure is maintained throughout the exposure period. Following the exposure period of 6 to 24 hours, depending on the degree of contamination and the penetrability of the package, the gas is exhausted and a vacuum of approximately 635 mm Hg is drawn. Filtered air is then introduced into the chamber until atmospheric pressure is attained. At temperatures lower than 55°C the exposure period will be much longer. Monitoring of the sterilization cycle shall require the employment of modern instrumentation to monitor the concentration of ETO, the temperature, the time and the moisture content in the sterilizing chamber.

B-2. BIOLOGICAL INDICATOR

B-2.1 Knowledge of the process may be gained by monitoring the physical parameters achieved in the sterilizer, and by the inclusion, in each sterilization cycle, of bacteriological test pieces distributed throughout the load. These usually consist of aluminium foil or polyethylene film or paper disk on which has been dried at least one million viable and potentially recoverable spores of *Bacillus subtilis* var. niger (NCTC 10073). It is recommended that at least 10 spore strips be used in each sterilization cycle. The approximate D_{10} value at 50 percent RH and 54°C may be about 3 or 1·7 minutes for ETO concentrations of 600 or 1 200 mg/L respectively.

B-3. RETENTION OF ETO

B-3.1 In ETO sterilization a note should be taken of absorption of the gas by some materials. For example, plastics exposed for 4 hours to 560 mg/L at 50 percent RH and 55°C may retain ETO as follows: PVC (1-3 percent), polystyrene (1·5-2 5 percent), PE (0·5-1 percent), polypropylene (1·5 percent), natural and synthetic rubber (2-3·5 percent), silicone rubber (1·5-2 percent). Some plastics (for example, acrylics, polystyrene) are affected by the fluorinated hydrocarbon mixtures, resulting in a crazing effect, evidenced by a milky discoloration, minute cracks or a crystal coating on the plastic.

B-4. AERATION

B-4.1 Great care must be taken to remove all traces of ETO after sterilization. This can be achieved by drawing sterile air through the load, the duration of aeration depending on the type of product (*see* Table 3).

TABLE 3 AERATION TIMES

(*Clause* B-4.1)

PRODUCTS	TEMPERATURE	RECOMMENDED AERATION TIME (h)
(1)	(2)	(3)
Glass, paper, and thin rubber articles	Room	24
Gum, rubber (thicker than 6 mm) and PE articles	Room	48
Polyvinyl chloride (PVC)	Room	168
All other articles except PVC	Room	96
All of these materials	50°-60°C in an aerator	8-12

B-5. RESIDUALS

B-5.1 There is also the possibility of formation of toxic substances (ethylene glycol and ethylene chlorohydrin) resulting from the exposure to the gas. This is particularly true for the use of ethylene oxide in sterilizing articles that contain moisture and chloride ions (as in medicated dressings). ETO sterilized products must therefore be monitored to assure that the residual gas and its reaction products do not exceed recommended limits as given in Table 4.

TABLE 4 MAXIMUM RESIDUE LIMITS FOR ETHYLENE OXIDE (ETO) ETHYLENE CHLOROHYDRIN (ETCH), AND ETHYLENE GLYCOL (ETG)

MEDICAL DEVICE	ETO*	ETCH* (ppm)	ETG
(1)	(2)	(3)	(4)
Implant			
Small (< 10 g)	250	250	5 000
Medium (10-100 g)	100	100	2 000
Large (> 100 g)	25	25	500
Intraoccular lenses	25	25	500
Surgical scrub sponges	25	250	500
Devices			
Contacting mucosa and skin	250	250	5 000
Contacting blood (ex-vivo)	25	25	250
Intrauterine	5	10	10

*Induce base-pair substitution mutation.

APPENDIX C

(*Clause* 5.2)

MEDIA FOR TESTS

C-1. FLUID THIOGLYCOLLATE MEDIUM

C-1.1 Composition

L-cystine	0·5 g
Sodium chloride	2·5 g
Dextrose ($C_6H_{12}O_6.H_2O$)	5·5 g
Agar granulated (moisture contents not in excess of 15 percent)	0·75 g
Yeast extract (water soluble)	5·0 g
Pancreatic digest of casein	15·0 g
Sodium thioglycollate or Thioglycollic acid	0·3 ml
Resazurin sodium solution (1 in 1 000) freshly prepared	1·0 ml
Water	1 000 ml
*p*H after sterilization	7·1 ± 0·2

C-1.2 Mix in the order named, the L-cystine, sodium chloride, dextrose, agar, water soluble yeast extract, and pancreatic digest of casein, in a mortar with thorough grinding. Stir in a small quantity of heated water, transfer to a suitable container, with repeated washing of the mortar, add the remainder of 1 000 ml of water, and heat until solution is effected. Dissolve the sodium thioglycollate or thioglycollic acid in the solution and, if necessary, adjust the solution with aqueous sodium hydroxide (4 percent w/v) so that, after sterilization, it will have a *p*H of 7·1 ± 0·2. If filtration is necessary, heat the solution again without boiling, and filter while hot through moistened filter paper. Add the resazurine sodium solution, mix, and place the medium in suitable vessels which provide a ratio of surface to depth of medium such that not more than the upper half of the medium has undergone a colour change indicative of oxygen uptake at the end of the incubation period. Sterilize in an autoclave (*see* **4.1**). Do not use this medium if it is evaporated to an extent affecting its fluidity. If more than the upper

one third has acquired a pink colour, the medium may be restored once by heating on asteam bath or in free flowing steam until the pink colour disappears. When ready for use, approximately the upper one-tenth of the medium should have a pink colour. Use fluid thioglycollate medium by incubating it *under aerobic* conditions.

C-2. ALTERNATIVE THIOGLYCOLLATE MEDIUM FOR DEVICES HAVING TUBES WITH SMALL LUMINA

C-2.1 Composition

L-cystine	0.5 g
Sodium chloride	2·5 g
Dextrose ($C_6H_{12}O_6.H_2O$)	5·5 g
Yeast extract (water soluble)	5·0 g
Pancreatic digest of casein	15·0 g
Sodium thioglycollate	0·5 g
or thioglycollic acid	0·3 ml
Water	1 000 ml
*p*H after sterilization	7·1 ± 0·2

C-2.2 Heat the ingredients in a suitable container until solution is effected. Mix and, if necessary, adjust the solution with aqueous sodium hydroxide (4 percent w/v) so that, after sterilization, it will have a *p*H of 7·1 ± 0·2. Filter, if necessary, place in suitable vessels, and sterilize in an autoclave (*see* **4.1**). The medium is freshly prepared or heated, in a steam bath and allowed to cool just prior to use. Do not repeat.

C-2.3 Use alternative thioglycollate medium in a manner that will assure anaerobic conditions for the duration of the incubation period.

C-3. SOYABEAN — CASEIN DIGEST MEDIUM

C-3.1 Composition

Pancreatic digest of casein	17·0 g
Papaic digest of soyabean meal	3·0 g
Sodium chloride	5·0 g
Disabic potassium phosphate	2·5 g
Dextrose ($C_6H_{12}O_6.H_2O$)	2·5 g
Water	1 000 ml
*p*H after sterilization	7·3 ± 0·2

C-3.2 Dissolve the solids in the water, warming slightly to effect solution. Cool the solution to room temperature, and adjust with aqueous sodium hydroxide (4 percent w/v), if necessary, and dispense into suitable vessels. Sterilize in an autoclave (*see* **4.1**). Use soyabean casein digest medium by incubating it *under aerobic* conditions.

APPENDIX D

(*Clause* 5.3.4)

TEST PROCEDURE FOR PURIFIED COTTON GAUZE, SURGICAL DRESSING AND RELATED ARTICLES, SUTURES, STERILIZED DEVICES, AND PETROLATUM GAUZE

D-1. PURIFIED COTTON, GAUZE, SURGICAL DRESSINGS & RELATED ARTICLES

D-1.1 From each packet of cotton, rolled gauze or gauze bandage being tested, remove with sterile instruments two or more portions of 100 mg to 500 each from the innermost part of the sample. From individually packaged single-use materials such as gauze pads, remove a single portion of 250 to 500 mg or the entire article in the case of small, that is 25 × 75 mm or smaller, adhesive absorbent bandages. Aseptically transfer these portions of the article to the specified number of containers of appropriate media.

D-2. SUTURES

D-2.1 Place the container in a suitable antimicrobial solution containing crystal violet or another suitable dye, for not less than 3 hours. Remove with sterile forceps and if the container shows no evidence of leakage, hold under aseptic conditions prior to testing. Open the container, aseptically, and with sterile instruments transfer sutures to containers of appropriate media.

D-3. STERILIZED DEVICES

D-3.1 The following considerations apply to sterilized devices manufactured in lots, each consisting of a number of units. Special considerations apply to sterile devices manufactured in small lots or in individual units where the self-destructive nature of the sterility test renders the conventional sterility test impracticable. For these articles appropriate and acceptable modifications to the sterility test must be made.

D-3.2 For articles of such size and shape as to permit complete immersion is not more than 1 000 ml of culture medium, test the intact article, using the appropriate media, and incubate as directed under **5.4.**

D-3.3 For devices having hollow tubes, such as transfusion or infusion assemblies, or where the size of an item makes immersion impracticable, and only the fluid pathway must be sterile, flush the lumen of each of 20 units with a sufficient quantity of fluid thioglycollate medium and the lumen of each of 20 units with a sufficient quantity of soyabean casein digest medium to yield a recovery of not less than 15 ml of each medium and incubate with not less than 100 ml of each of the two media as directed under **5.3.4.** For devices in which the lumen is so small that fluid thioglycollate medium will not pass through substitute alternative thioglycollate medium for fluid thioglycollate medium and incubate that inoculated medium anaerobically.

D-3.4 Where the entire intact article cannot be tested for sterility because of its size and shape, by impression in not more than 1 000 ml of culture medium expose that portion of the article most difficult to sterilize and test that portion or where practicable remove two or more portions each from the innermost portion of the article. Aseptically transfer these portions of the article to the specified number of vessels of appropriate media in a volume of not more than 1 000 ml and incubate as directed under **5.4.**

D-3.5 Where the presence of the test specimen in the medium interferes with the test because of bacteriostatic or fungistatic action, rinse the article thoroughly with a minimal amount of rinse fluid (*see* diluting and rinsing fluids under test procedure using membrane filtration under Appendix E). Recover the rinse fluid, and test as directed for devices under test procedures using membrane filtration (*see* **5.4**).

D-4. PETROLATUM GAUZE

D-4.1 Prepare fluid thioglycollate medium as directed except to use 1·0 g of agar and to add 5·0 g of gelatin for each litre of medium and test as described under Growth Promotion Test (*see* **5.3**).

D-4.2 Place 300 ml of medium in suitable wide-mouth containers of about 500 ml capacity. Loosely close each jar with an aluminium lid without a liner but with a rubber ring gasket fitted to the inside circumference of the lid, and secure with a muslin cover during sterilization and until ready to use. Sterilize in an autoclave (*see* **4.1**).

D-4.3 To the medium warmed to 52° transfer aseptically the entire contents of a single package of the petrolatum gauze to be tested. Close tightly and shake each container in a reciprocating shaker for 10 minutes. Cool the container in a slanting position until the petrolatum

forms a solid seal over the surface of the medium. Break the petrolatum seal by a single quick shake. Incubate at 20° to 25° for not less than 7 days, then shake in a reciprocating shaker for 10 minutes. Transfer aseptically 0·5 ml of the product medium mixture to 15 ml of culture medium. Incubate at 30° to 35°C for not less than 7 days.

APPENDIX E

(*Clauses* 5.4 *and* D-3.5)

TEST PROCEDURE USING MEMBRANE FILTRATION TECHNIQUE

E-1. APPARATUS

E-1.1 A suitable unit consists of a closed reservoir and a receptacle between which a properly supported membrane or membranes of appropriate porosity are placed. A membrane generally suitable for sterility testing has a nominal porosity of 0·45 ± 0·02 μm, a diameter of approximately 47 mm, and a flowrate of 55 to 75 ml of water per minute at a pressure of 70 mm of mercury. The entire unit may be assembled and sterilized with the membrane(s) in place prior to use in the test or the membranes may be sterilized separately by steam under pressure, or by any method (for example, by radiation if the membrane is radiation stable) that yields proper performance. Where the article to be tested is an oil, sterilize the membrane separately, and after thorough drying, assemble the unit, using aseptic precautions.

E-2. DILUTING AND RINSING FLUIDS

E-2.1 Fluid A — Dissolve 1 g of peptic digest of animal tissue (equivalent to DIFCO grade) in water to make 1 litre, filter or centrifuge to clarify, adjust to a *p*H of 7·1 ± 0·2, dispense into containers in 100 ml quantities and sterilize in an autoclave (*see* **4.1**).

E-2.2 Fluid D — If the test specimen contains lecithin or oil, use Fluid *A* to each litre of which has been added 1 ml of polysorbate 80, adjust to *p*H 7·1 ± 0·2, dispense into flasks and sterilize in an autoclave (*see* **4.1**).

NOTE — A sterile fluid must not have antibacterial or antifungal properties if it is to be considered suitable for dissolving, diluting or rinsing an article under test for sterility.

E-3. DEVICES

E-3.1 Devices that are required to contain sterile pathways may be tested for sterility by the membrane filtration technique as given in **E-3.1.1**.

E-3.1.1 Aseptically pass a sufficient volume of Fluid *D* through each of not less than 20 devices so that not less than 100 ml is recovered from each device. Collect the fluids in aseptic containers and filter the entire volume collected through membrane filter funnel(s). One or two membranes may be used for the entire number of containers prescribed. If the product is bacteriostatic or fungistatic, rinse the membranes with three 100 ml portions of Fluid *A*. Aseptically remove the membrane(s) from the holder, cut the membrane in half, if only one is used, immerse the membrane or one-half of the membrane, in 100 ml of soyabean casein digest medium, and incubate at 20° to 25°C for not less than 7 days. Similarly immerse the other membrane or other half of the membrane in 100 ml of fluid thioglycollate medium, and incubate at 30° to 35°C for not less than 7 days. Where the devices are large, and lot sizes are small test an appropriate number of units as described for similar cases under test procedures for direct transfer to test media (*see also* **5.3.6**).

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TO

IS:10150-1981 GUIDE FOR STERILIZATION OF MEDICAL PRODUCTS

Corrigenda

(Page 22, clause D-3.2):

a) line 2 - Substitute 'in' *for* 'is'.

b) line 3 - Substitute '5.3.4' *for* '5.4'.

(Page 22, clause D-3.4, last line) - Substitute '5.3.4' *for* '5.4'.

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(*Page 8, clause 4.2.1*) - Add the following new clauses after 4.2.1 and renumber the subsequent clauses accordingly:

'4.3 Sterilization by Formaldehyde Gas - The material to be sterilized is exposed to formaldehyde gas at low temperature (approximately 55°C). The process is carried out in a specialised vessel. The process depends on four factors, namely, temperature, relative humidity, gas concentration and exposure time. Besides, the product design which includes the penetrability, the packaging system employed, bioburden of the product and the state of hydration of the micro-organisms is also important.

4.3.1 *Bioburden* - A bioburden history of the product shall be established since this factor influences the effectiveness of the sterilization process.

4.3.2 *Packaging System* - The packaging materials shall be highly permeable to formaldehyde gas or alternatively, a breathing system may be provided which allows the penetration of the formaldehyde gas into the product. The material such as double thickness muslin or paper or single thickness polyethylene (PE) or polypropylene, laminates as cello-PE, mylar-PE, foil-PE may be used.

4.3.3 *Sterilization Process* - Formaldehyde gas sterilization requires a specialised equipment designed for this particular purpose. The instructions provided by the manufacturer of the formaldehyde gas sterilizer shall be followed. The following exposure conditions shall be achieved for the formaldehyde gas sterilization:

Temperature - 55°C ± 2°C

Vacuum - 700 mm ± 20 mm before gas entry in the chamber and 600 mm ± 20 mm during the sterilization process

Relative humidity - 55 to 60 percent

Exposure time - 10 - 12 hours

Gas concentration in the chamber - 400 mg - 500 mg per litre

The above cycle shall be monitored by a recording system which may be manual or automatic.

4.3.4 *Biological Indicator* - The biological indicators are required for the purpose of determination of sterility. Biological indicators shall be used to monitor effectiveness of the sterilization of the product. At least 10 spore strips shall be used of 10^6 *Bacillus subtilis var niger* (NCTC 10073) for each sterilizing cycle.'

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